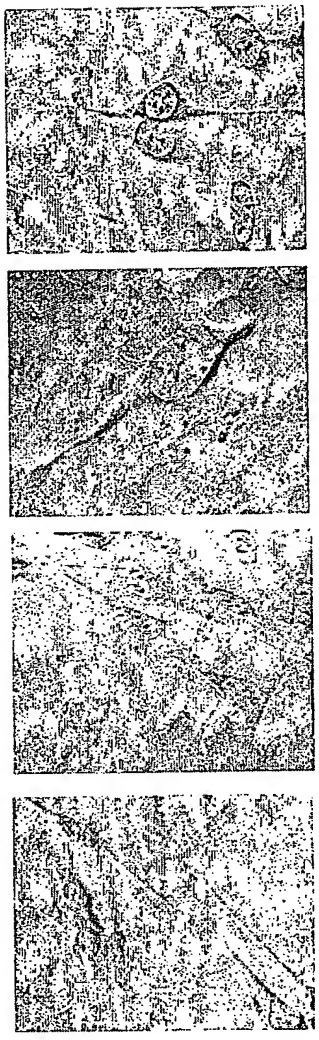

Figure 1

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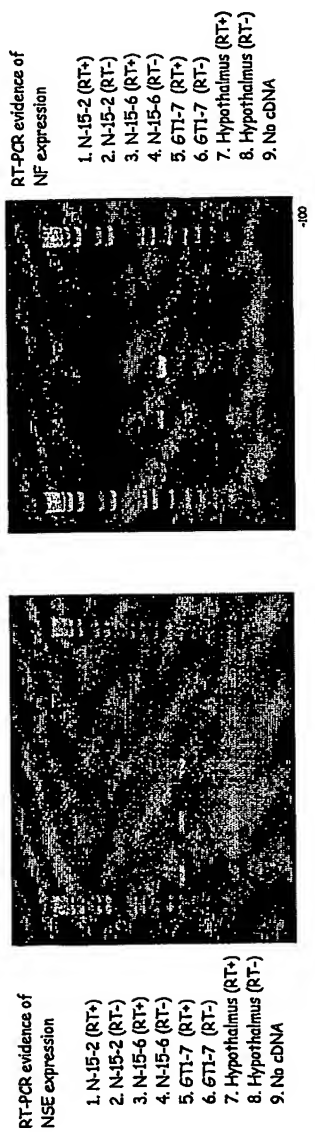
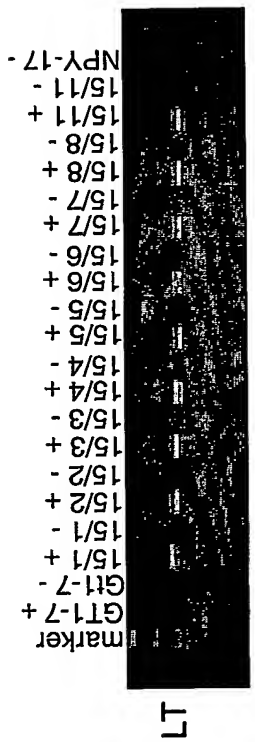

RT-PCR evidence of NSE expression
M 1 2 3 4 5 6 7 8 9 M
1. N-15-2 (RT+)
2. N-15-2 (RT-)
3. N-15-6 (RT+)
4. N-15-6 (RT-)
5. 6T1-7 (RT+)
6. 6T1-7 (RT-)
7. Hypothalmus (RT+)
8. Hypothalmus (RT-)
9. No cDNA
RT-PCR evidence of NF expression
M 1 2 3 4 5 6 7 8 9 M
1. N-15-2 (RT+)
2. N-15-2 (RT-)
3. N-15-6 (RT+)
4. N-15-6 (RT-)
5. 6T1-7 (RT+)
6. 6T1-7 (RT-)
7. Hypothalmus (RT+)
8. Hypothalmus (RT-)
9. No cDNA
100

Figure 2


LT
marker
GT1-7 +
Gt1-7 -
15/1 +
15/1 -
15/2 +
15/2 -
15/3 +
15/3 -
15/4 +
15/4 -
15/5 +
15/5 -
15/6 +
15/6 -
15/7 +
15/7 -
15/8 +
15/8 -
15/11 +
15/11 -
NPY-17 -

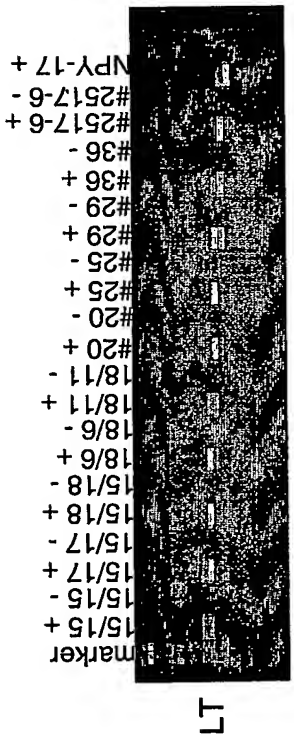
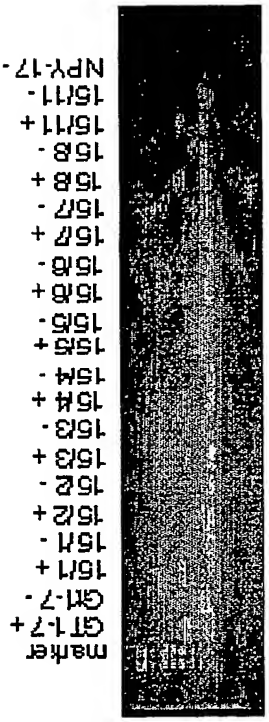

LT
marker
15/15 +
15/15 -
15/17 +
15/17 -
15/18 +
15/18 -
18/6 +
18/6 -
18/11 +
18/11 -
#20 +
#20 -
#25 +
#25 -
#29 +
#29 -
#36 +
#36 -
#2517-6 +
#2517-6 -
NPY-17 +

Figure 3A


marker
GT1-7+
GM1-7-
15/1+
15/1-
15/2+
15/2-
15/3+
15/3-
15/4+
15/4-
15/5+
15/5-
15/6+
15/6-
15/7+
15/7-
15/8+
15/8-
15/11+
15/11-
NPY-17-
NSE

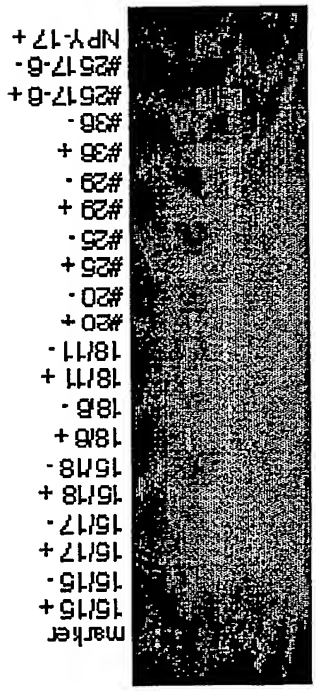

marker
15/15+
15/15-
15/17+
15/17-
15/18+
15/18-
18/8+
18/8-
18/11+
18/11-
#20+
#20-
#25+
#25-
#29+
#29-
#36+
#36-
#2517-8+
#2517-8-
NPY-17+
NSE

Figure 3B

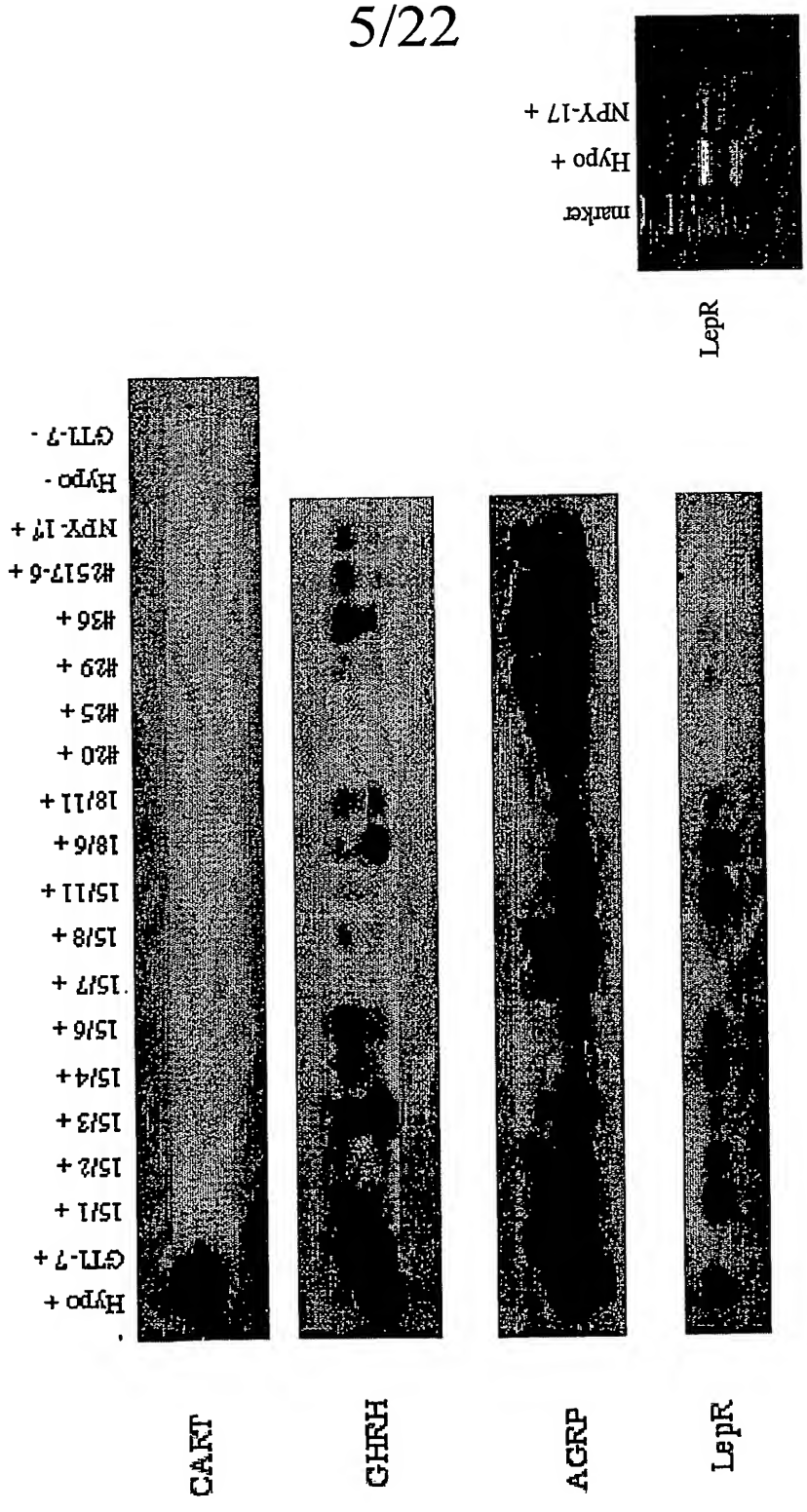

CART
GHRH
AGRP
LepR
Hypo +
GTT-7 +
15/1 +
15/2 +
15/3 +
15/4 +
15/6 +
15/7 +
15/8 +
15/11 +
18/6 +
18/11 +
#20 +
#25 +
#29 +
#36 +
#2517-6 +
NPY-17 +
Hypo -
GTT-7 -
LepR
marker
Hypo +
NPY-17 +

Figure 4

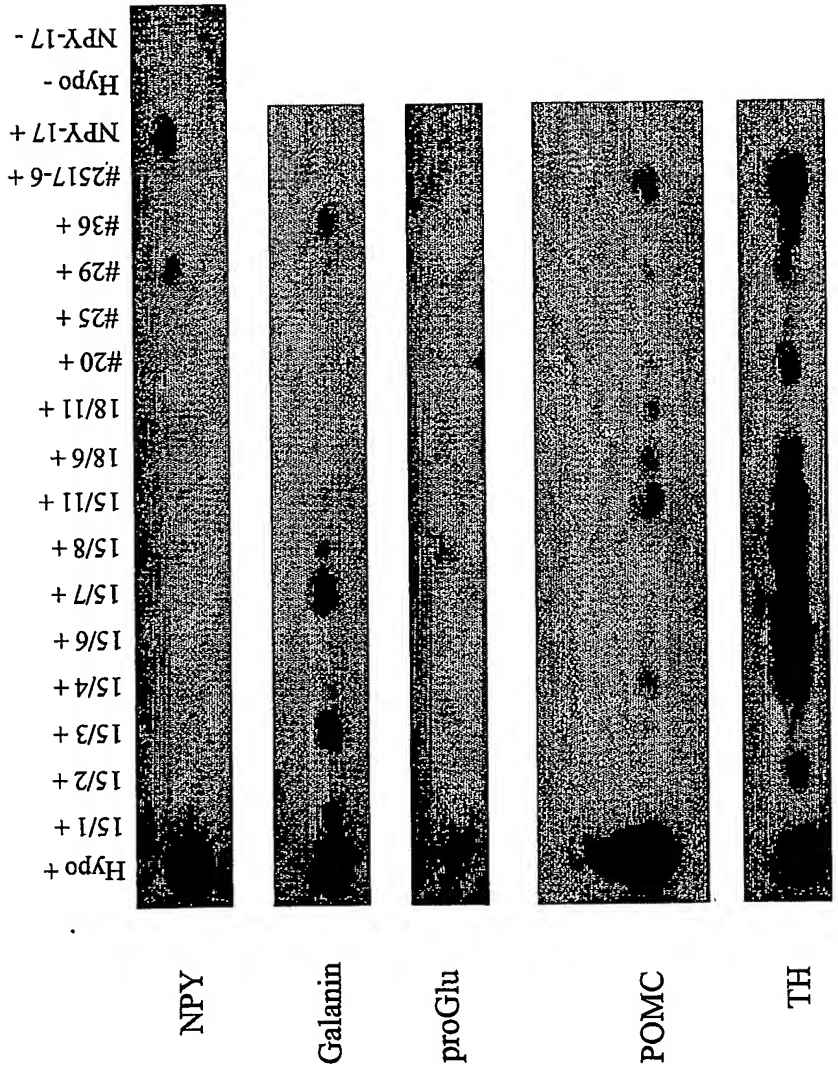

Hypo +
15/1 +
15/2 +
15/3 +
15/4 +
15/6 +
15/7 +
15/8 +
15/11 +
18/6 +
18/11 +
#20 +
#25 +
#29 +
#36 +
#2517-6 +
NPY-17 +
Hypo -
NPY-17 -
NPY
Galanin
proGlu
POMC
TH

Figure 5

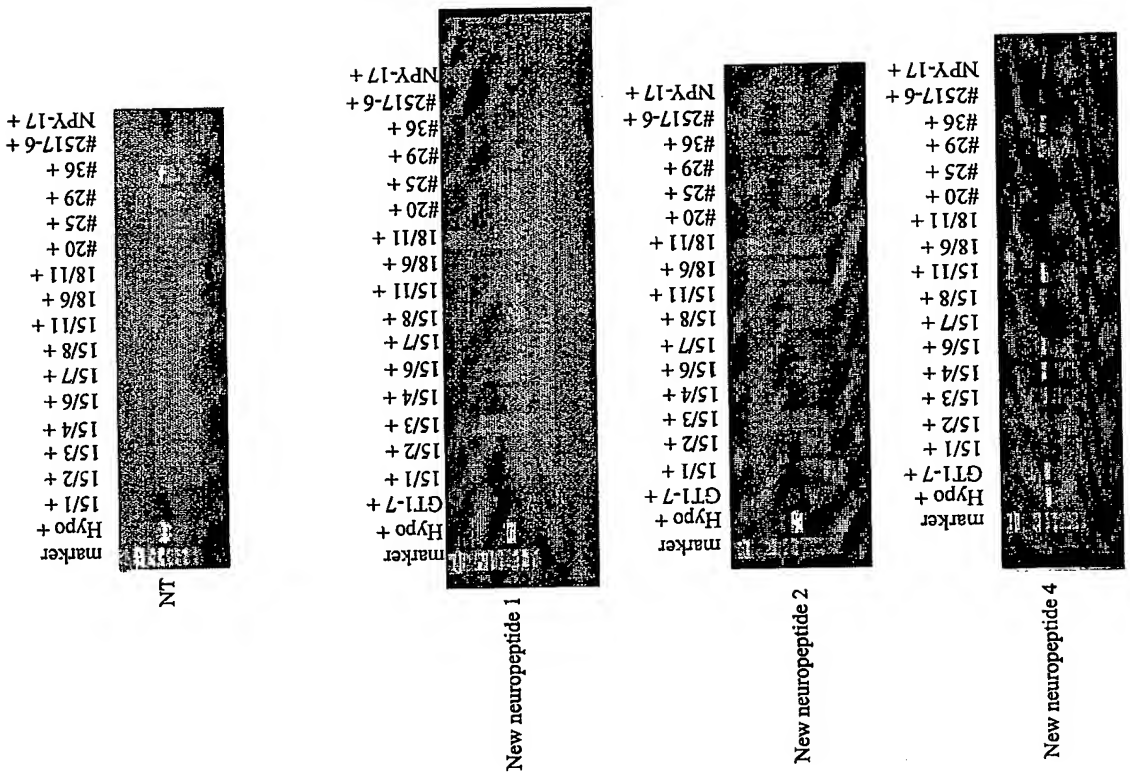
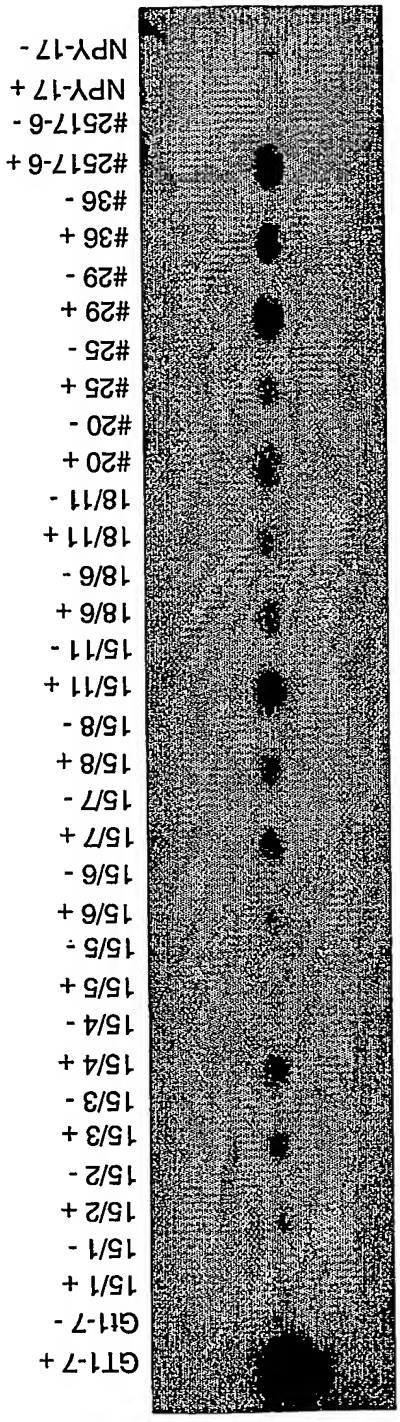

marker
Hypo +
GTI-7 +
15/1 +
15/2 +
15/3 +
15/4 +
15/6 +
15/7 +
15/8 +
15/11 +
18/6 +
18/11 +
#20 +
#25 +
#29 +
#36 +
#2517-6 +
NPY-17 +
New neuropeptide 4
New neuropeptide 2
New neuropeptide 1
LN

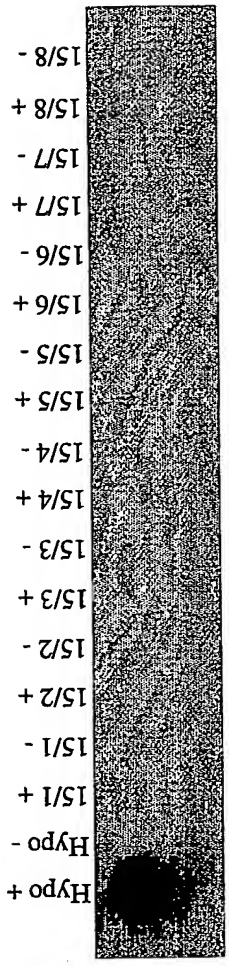
Figure 6


GnRH
GT1-7 +
Gt1-7 -
15/1 +
15/1 -
15/2 +
15/2 -
15/3 +
15/3 -
15/4 +
15/4 -
15/5 +
15/5 -
15/6 +
15/6 -
15/7 +
15/7 -
15/8 +
15/8 -
15/11 +
15/11 -
18/6 +
18/6 -
18/11 +
18/11 -
#20 +
#20 -
#25 +
#25 -
#29 +
#29 -
#36 +
#36 -
#2517-6 +
#2517-6 -
NPY-17 +
NPY-17 -

G2R
Hypo +
Hypo -
15/1 +
15/1 -
15/2 +
15/2 -
15/3 +
15/3 -
15/4 +
15/4 -
18/6 +
15/4 -
18/6 -
15/5 +
15/5 -
15/6 +
15/6 -
15/7 +
15/7 -
15/8 +
15/8 -
15/11 +
15/11 -
18/11 +
18/11 -
#20 +
#20 -
#25 +
#25 -
#29 +
#29 -
#36 +
#36 -
#2517-6 +
#2517-6 -
NPY-17 +
NPY-17 -

Figure 7

10/511591



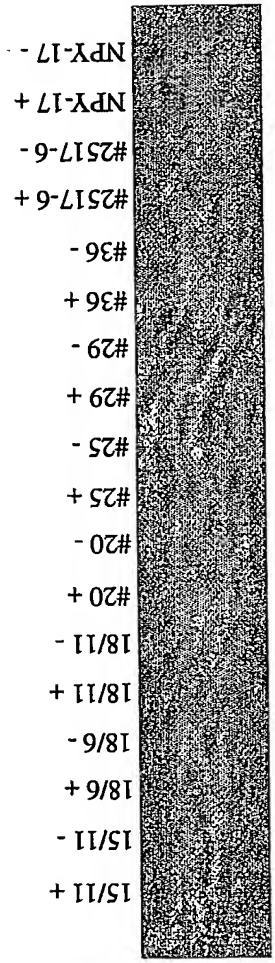


Figure 8

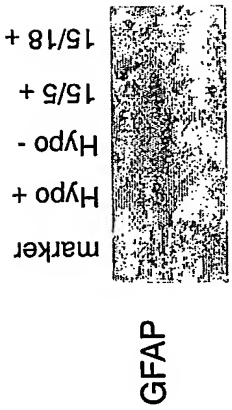


Figure 9

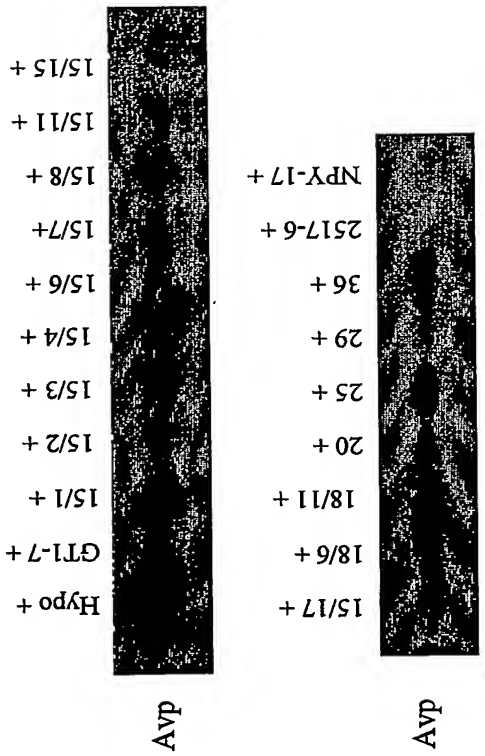
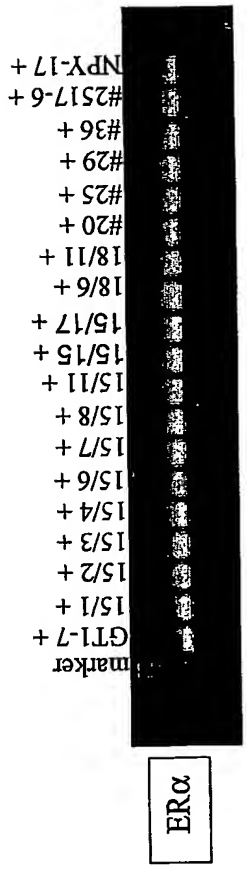

Avp
15/17 +
18/6 +
18/11 +
20 +
25 +
29 +
36 +
2517-6 +
NPY-17 +
Avp
Hypo +
GT1-7 +
15/1 +
15/2 +
15/3 +
15/4 +
15/6 +
15/7+
15/8 +
15/11 +
15/15 +

Figure 10


ERα
marker
GT1-7 +
15/1 +
15/2 +
15/3 +
15/4 +
15/6 +
15/7 +
15/8 +
15/11 +
15/15 +
15/17 +
18/6 +
18/11 +
#20 +
#25 +
#29 +
#36 +
#2517-6 +
NPY-17 +

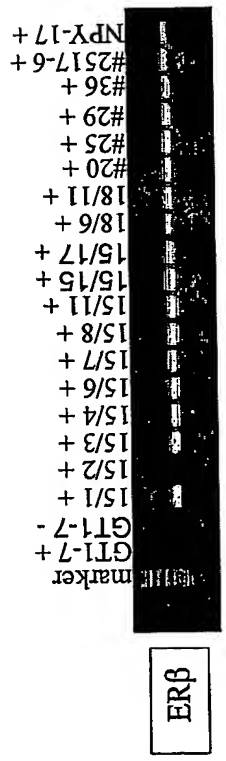

ERβ
marker
GT1-7 +
GT1-7 -
15/1 +
15/2 +
15/3 +
15/4 +
15/6 +
15/7 +
15/8 +
15/11 +
15/15 +
15/17 +
18/6 +
18/11 +
#20 +
#25 +
#29 +
#36 +
#2517-6 +
NPY-17 +

Figure 11

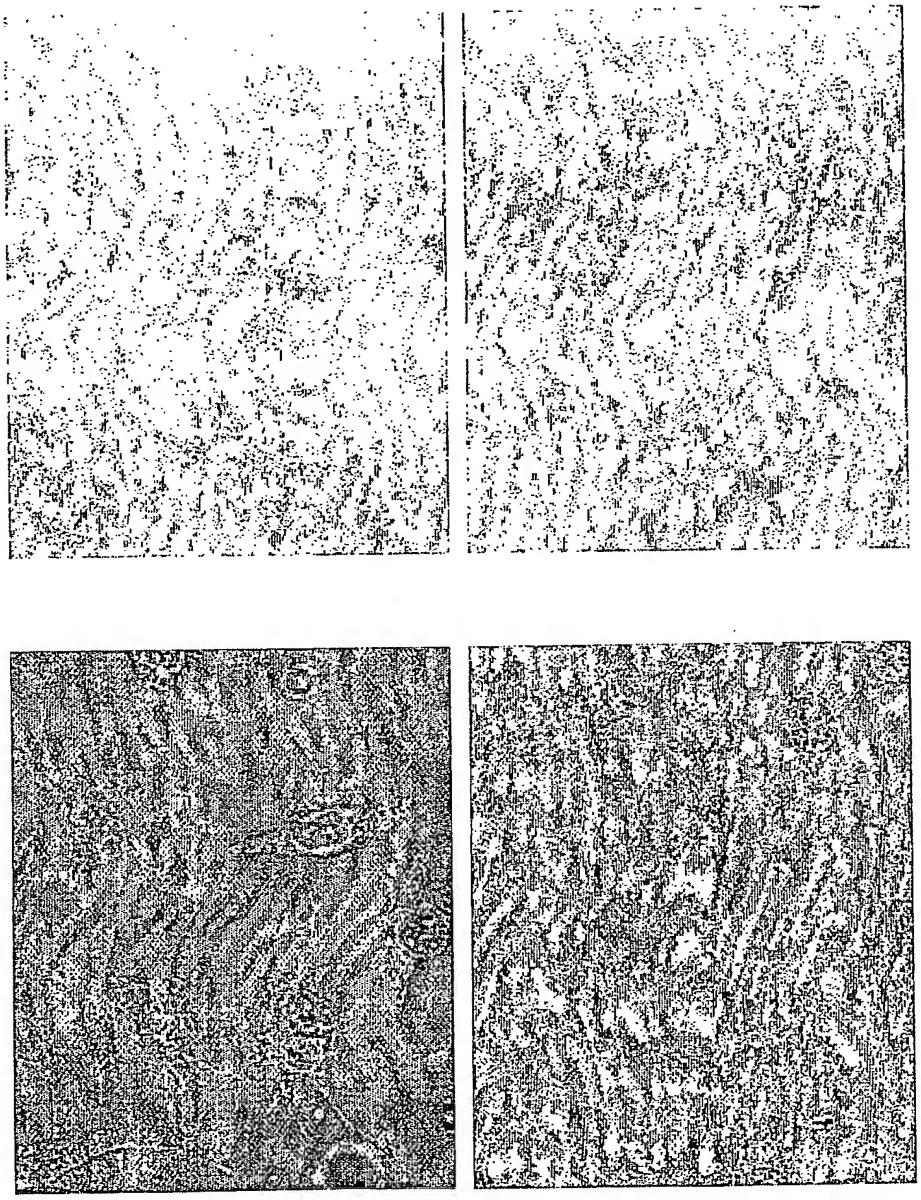


Figure 12

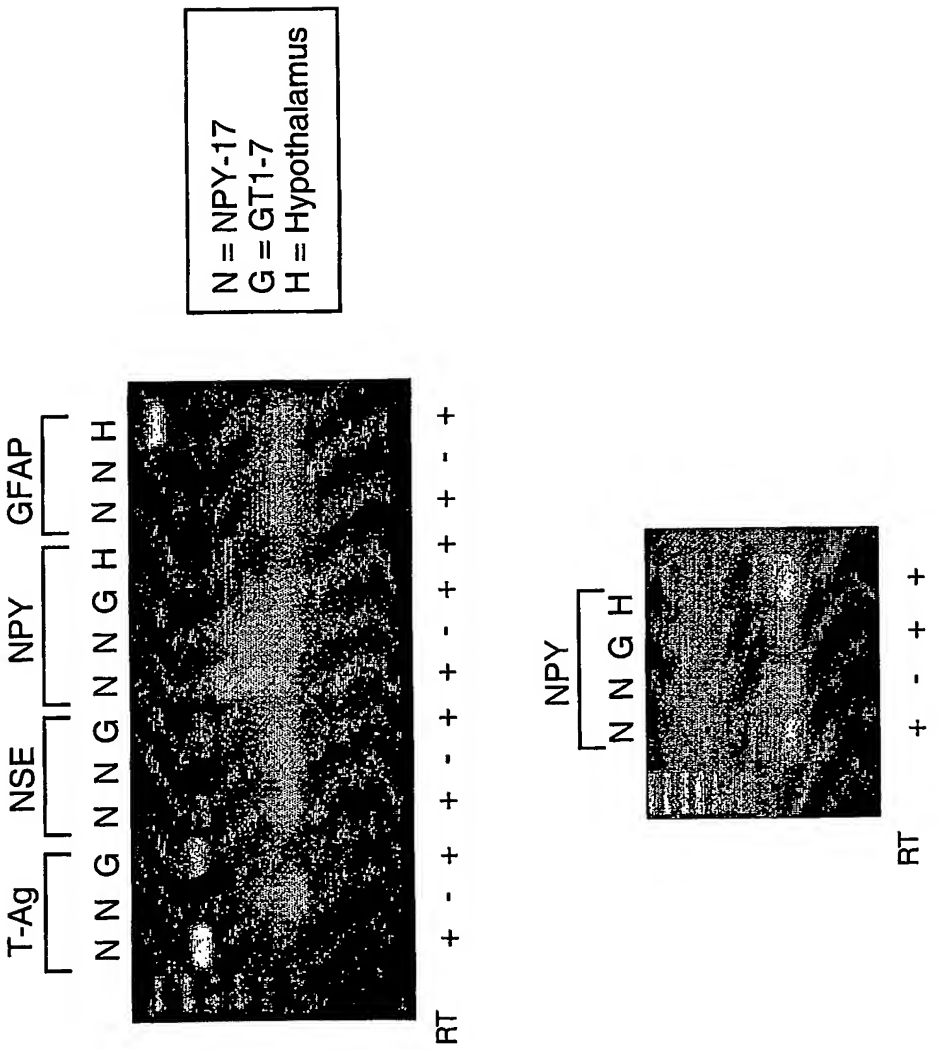

N = NPY-17
G = GT1-7
H = Hypothalamus
T-Ag
NSE
NPY
GFAP
N N G N N G N G H N N H
RT + - + + - + + - + + + -
NPY
N N G H
RT + - + +

Figure 13

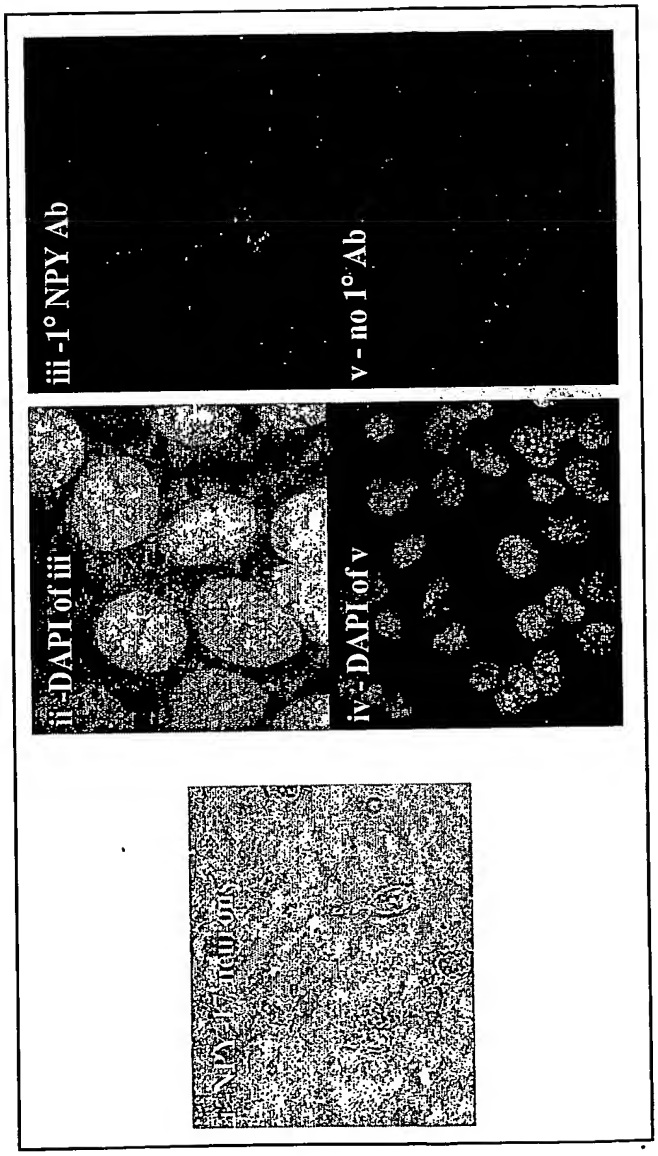


Figure 14

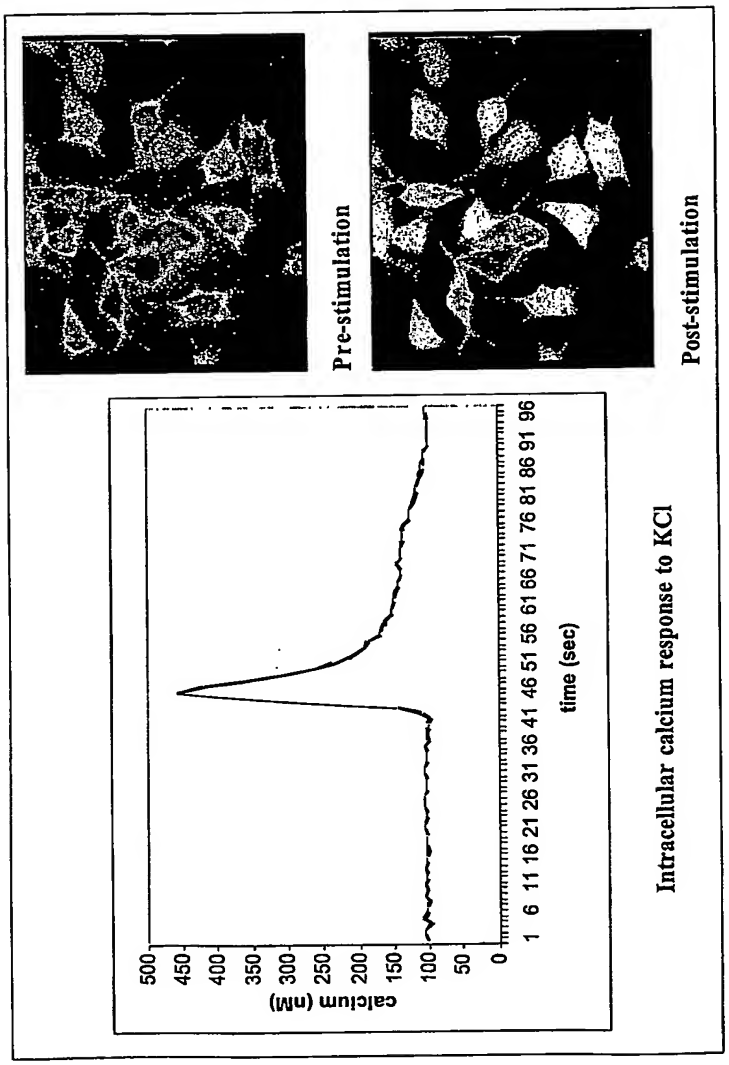

Pre-stimulation
Post-stimulation
Intracellular calcium response to KCl
calcium (nM)
500
450
400
350
300
250
200
150
100
50
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96
time (sec)

Figure 15

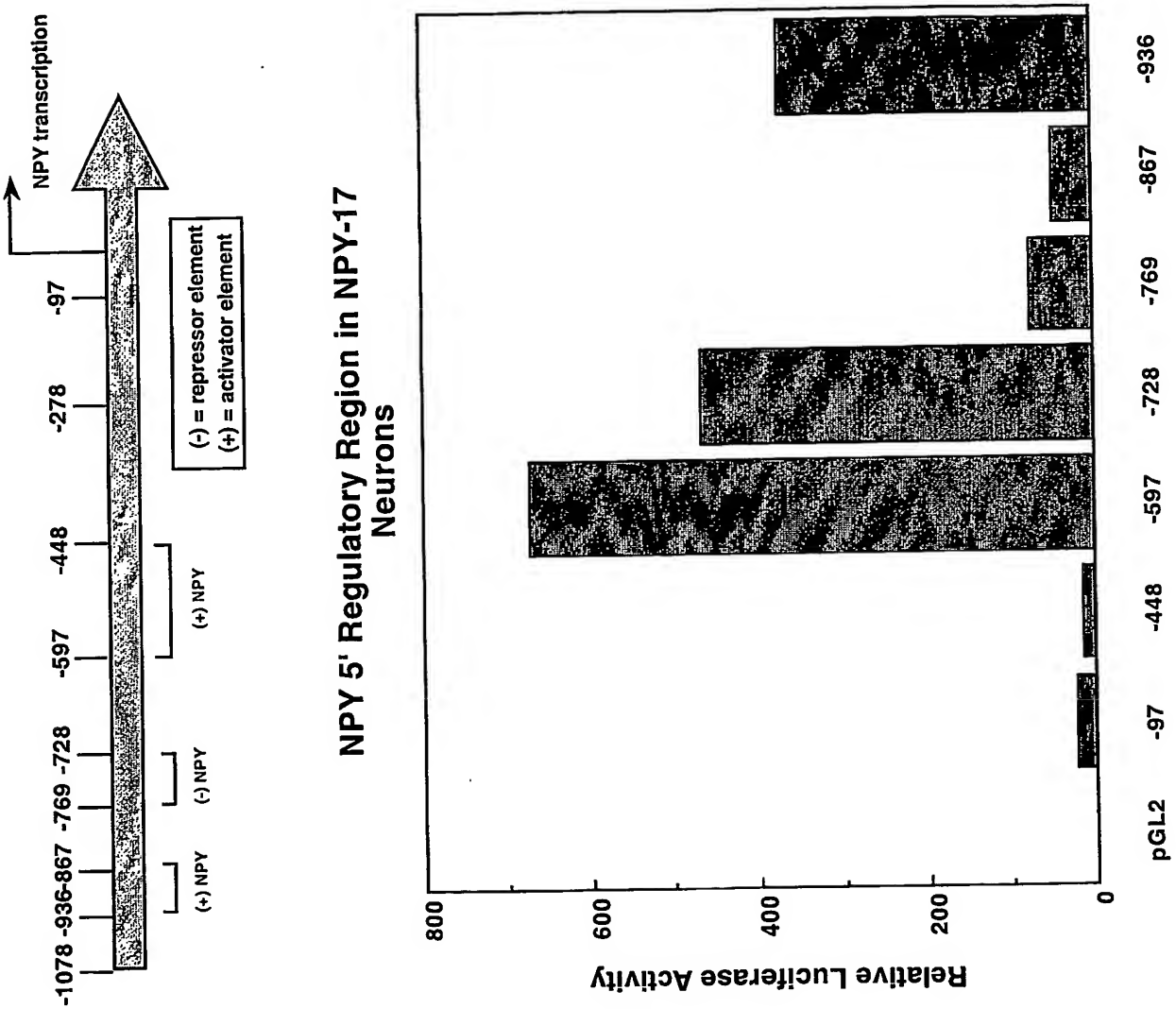

NPY transcription
-1078 -936 -867 -769 -728 -597 -448 -278 -97
(+) NPY
(-) NPY
(+) NPY
(-) = repressor element
(+) = activator element
NPY 5' Regulatory Region in NPY-17 Neurons
Relative Luciferase Activity
0
200
400
600
800
pGL2
-97
-448
-597
-728
-769
-867
-936

Figure 16

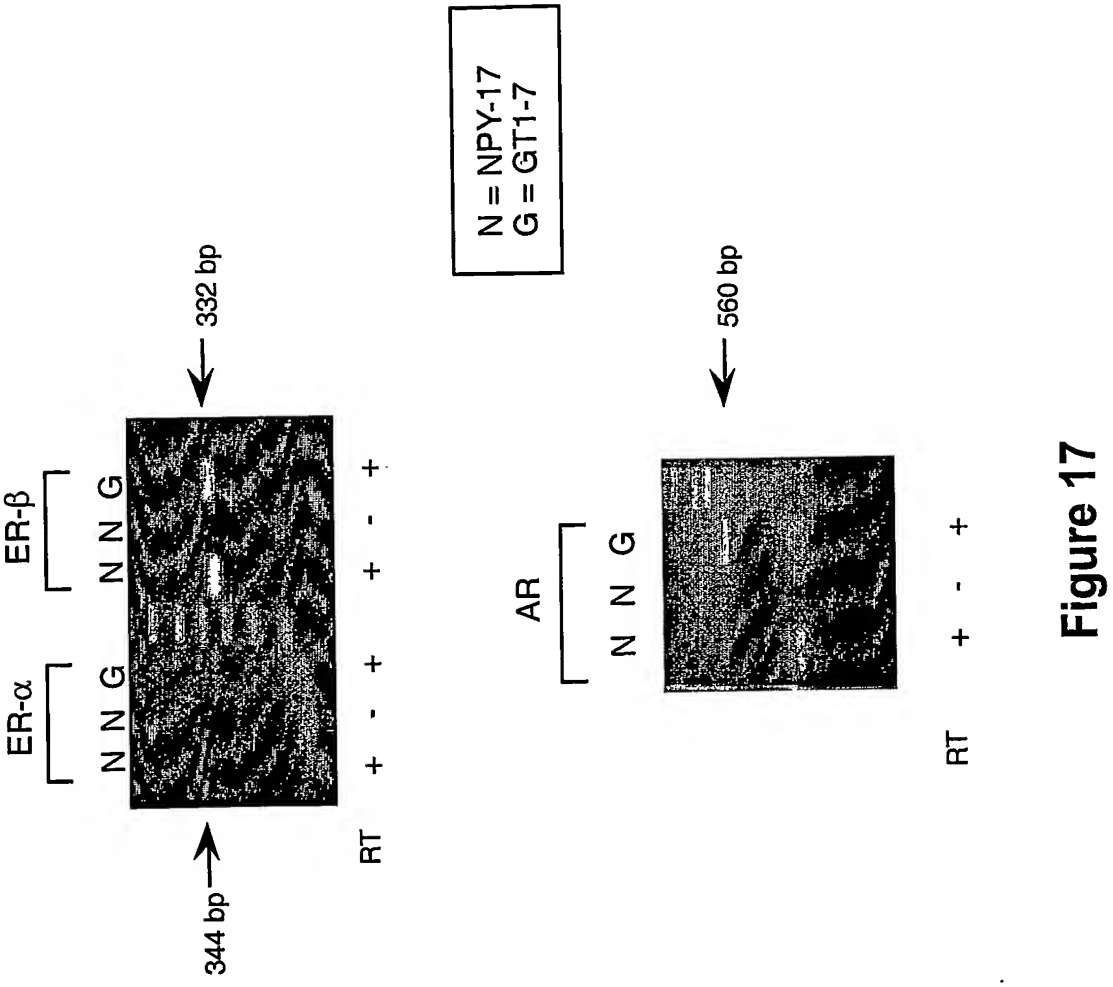


Figure 17

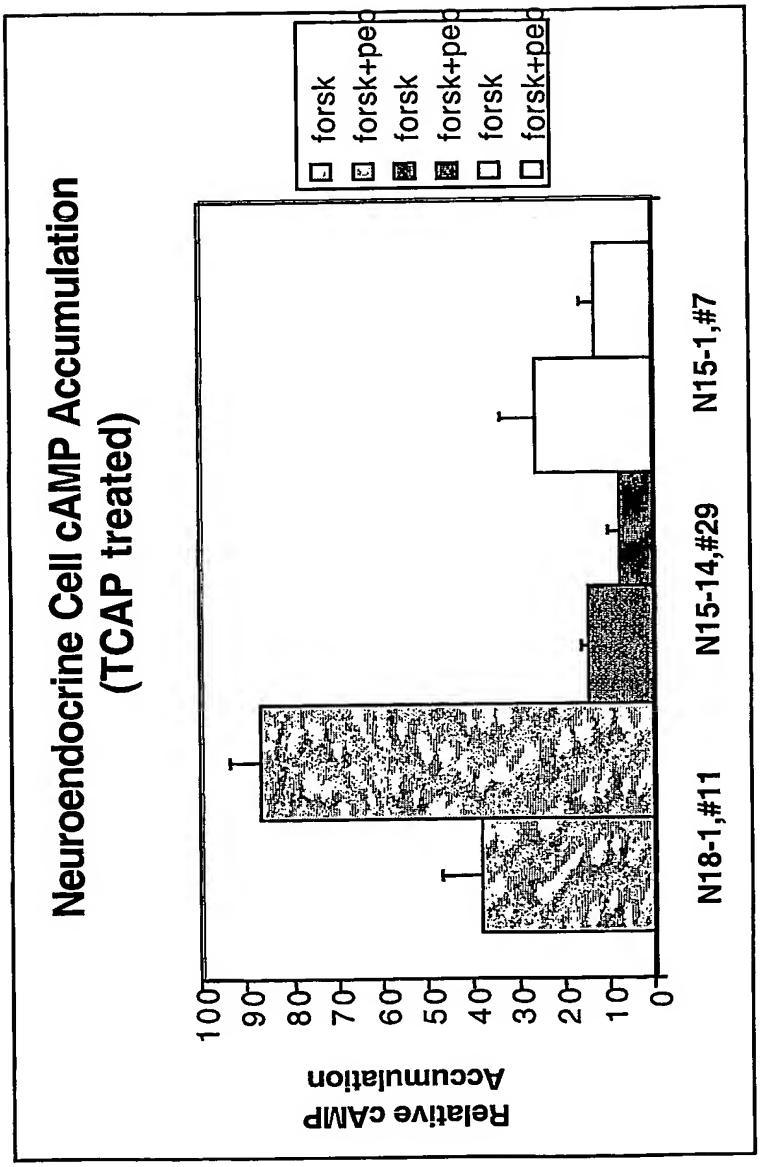

Neuroendocrine Cell cAMP Accumulation
(TCAP treated)
Relative cAMP
Accumulation
0
10
20
30
40
50
60
70
80
90
100
forsk
forsk+pe
forsk
forsk+pe
forsk
forsk+pe
N18-1,#11
N15-14,#29
N15-1,#7

Figure 18A

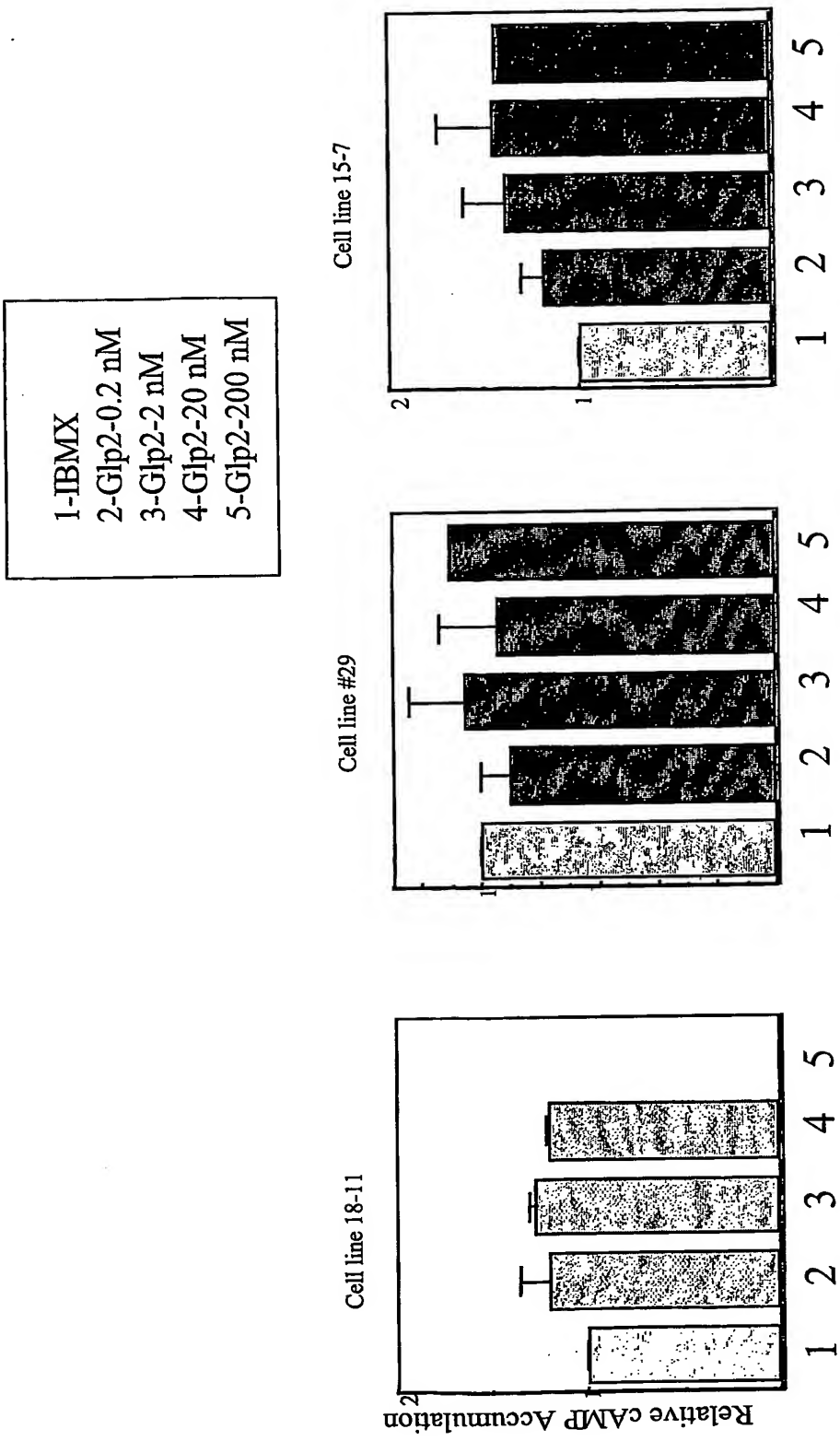

1-IBMX
2-Glp2-0.2 nM
3-Glp2-2 nM
4-Glp2-20 nM
5-Glp2-200 nM
Cell line 15-7
Cell line #29
Cell line 18-11
Relative cAMP Accumulation
1 2 3 4 5
2
1

Figure 18B

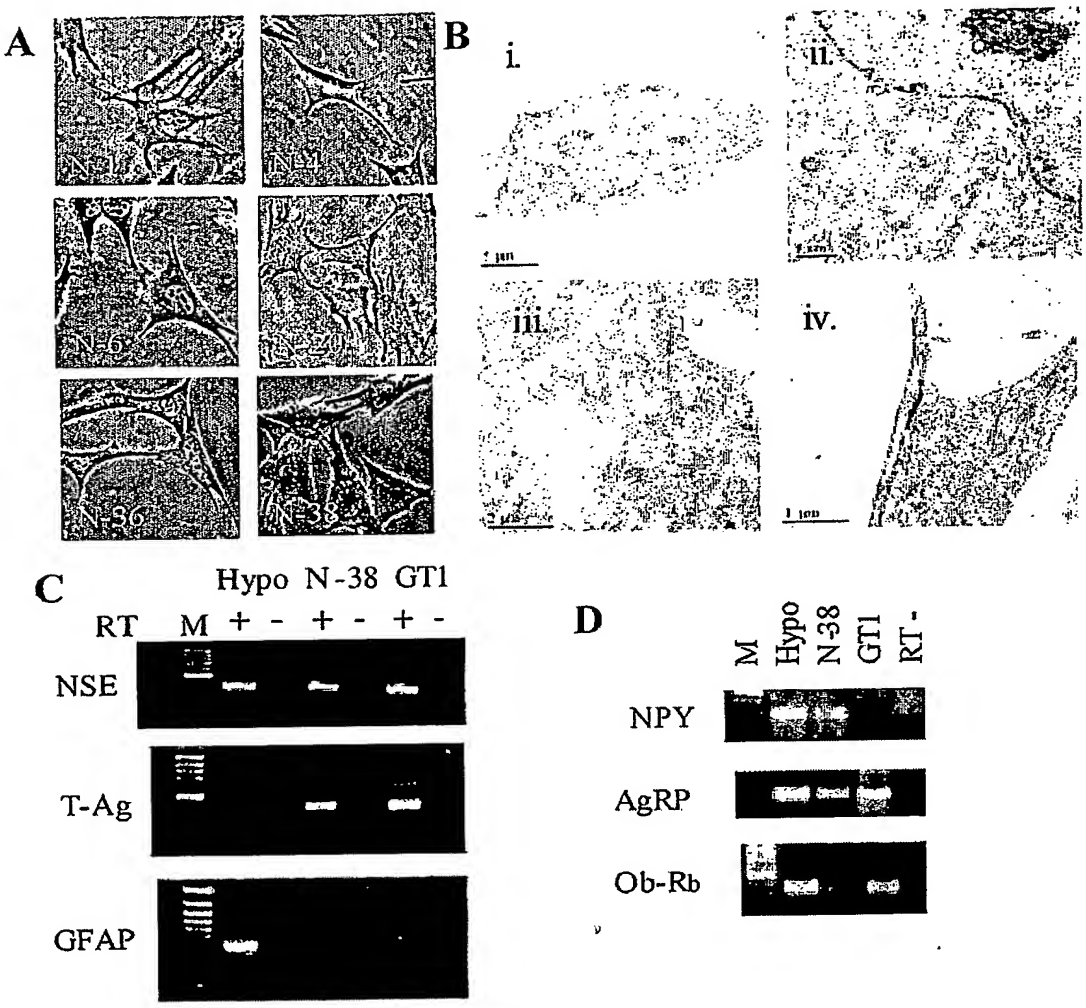
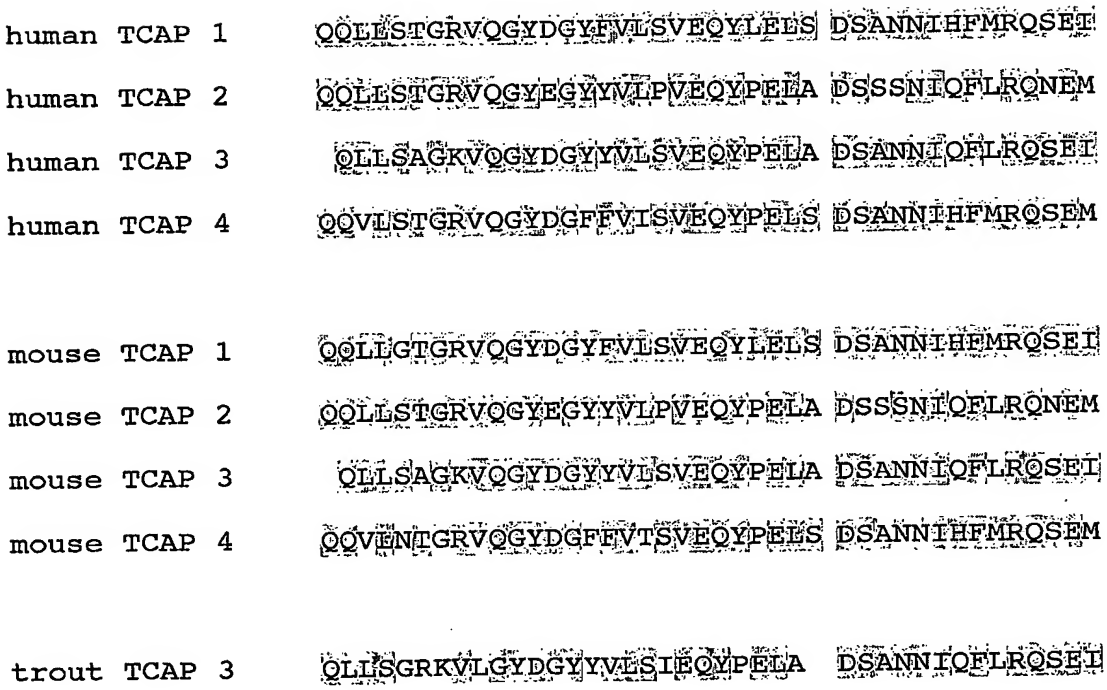

A
N-6
N-36
B
i.
ii.
iii.
iv.
C
Hypo N-38 GT1
RT M + - + - + -
NSE
T-Ag
GFAP
D
M Hypo N-38 GT1 RT -
NPY
AgRP
Ob-Rb

Figure 19

Figure 20


human TCAP 1 QQLLSTGRVQGYDGYFVLSVEQYLELS DSANNIHFMRQSET
human TCAP 2 QQLLSTGRVQGYEGYYVLPVEQYPELA DSSSNIQFLRQNEM
human TCAP 3 QLLSAGKVQGYDGYYVLSVEQYPELA DSANNIQFLRQSEI
human TCAP 4 QQVLSTGRVQGYDGFFVISVEQYPELS DSANNIHFMRQSEM
mouse TCAP 1 QQLLGTGRVQGYDGYFVLSVEQYLELS DSANNIHFMRQSEI
mouse TCAP 2 QQLLSTGRVQGYEGYYVLPVEQYPELA DSSSNIQFLRQNEM
mouse TCAP 3 QLLSAGKVQGYDGYYVLSVEQYPELA DSANNIQFLRQSEI
mouse TCAP 4 QQVENTGRVQGYDGFFVTSVEQYPELS DSANNIHFMRQSEM
trout TCAP 3 QLLSGRKVLGYDGYYVLSIEQYPELA DSANNIQFLRQSEI

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